Fatty Acid Synthesis in Adipose Tissue

II. ENZYMATIC SYNTHESIS OF BRANCHED CHAIN AND ODD-NUMBERED FATTY ACIDS

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(Received for publication, August 29, 1960)

Recent studies have done much to clarify the biosynthetic mechanism of normal long chain fatty acid synthesis (1–4). However, very little is known about the biogenesis of odd-numbered and branched long chain fatty acids. Odd-numbered fatty acids have been identified as minor components of ruminant milk (5), human milk (6), human blood (7), human sebum (8), rat phospholipids (9), and the lipids of the mold Sporideum bakeri (10). The branched long chain fatty acids (iso and anteiso acids) have been found in small amounts in butter fat (11), ewe milk fat (12), woolwax (13), and human sebum (8), human milk (6), and human blood (7).

Using the isolated perfused cow’s udder, James et al. (14) have found that odd-numbered fatty acids are formed by the condensation of propionate with acetate. Feller and Feist (15) have reported that slices from adipose tissue incorporate propionic acid-carbon-14 into long chain fatty acids as efficiently as acetate. On the other hand, Gerson (16) concluded from experiments with intravenously injected valeric acid-carbon-14 that valeric acid was not the direct precursor of the odd-numbered fatty acids present in the butter fat of the cow. In 1952, El-Shazly (17) demonstrated that micro-organisms in the rumen of sheep could deaminate amino acids to form C-4 and C-5 branched chain acids (isobutyric, isovaleric, and α-methylbutyric acids), and he suggested that these acids might be the precursors of the long chain iso and anteiso acids found in the milk fat of ruminants. In experiments with the isolated perfused cow’s udder, Verbeke et al. (18) found that isovaleric acid-carbon-14 is not incorporated into iso acids more efficiently than the normal acids. James et al. (16) reported that acetate-carbon-14 incubated with human whole blood is incorporated into all of the odd-numbered straight and branched chain fatty acids (7), but Gerson et al. (19) that acetate was incorporated into the anteiso acids more slowly than into normal acids.

In the previous paper (19), we have described a partially purified soluble enzyme system obtained from adipose tissue of the rat which synthesizes long chain fatty acids from acetyl coenzyme A and malonyl coenzyme A. This paper describes the biosynthesis of odd-numbered, iso and anteiso fatty acids from propionyl coenzyme A, isobutyryl coenzyme A, isocaproyl coenzyme A, isovaleryl coenzyme A, and α-methylbutyryl coenzyme A by this enzyme system. A preliminary report of these findings has been presented (20).

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EXPERIMENTAL PROCEDURE

Propionyl-CoA, isobutyryl-CoA, isocaproyl-CoA, isovaleryl-CoA, and α-methylbutyryl-CoA were synthesized by the mixed anhydride procedure of Wieland and Rueff (21). Malonyl-CoA-2-C14 was synthesized from malonic acid-2-C14 by the procedure of Trams and Brady (22) and had a specific activity of 1 mc per mmole.

The enzyme was prepared from epididymal adipose tissue of male Sprague-Dawley or Osborne-Mendel rats, and purified 49-fold as described in the previous paper (19).

Incubation Conditions—The complete fatty acid synthesizing system contained potassium phosphate buffer, pH 7.0, 100 μmoles; malonyl-CoA-2-C14, 0.38 to 0.40 μmole (specific activity, 1 μc per μmole); TPNH, 0.5 μmole; 2-mercaptoethanol, 2.5 μmole; enzyme, 1 to 2 units; and acetyl-CoA or another acyl-CoA acceptor, 0.20 to 0.24 μmole. The final volume was adjusted to 2 ml with water. After incubation at 30° for 1½ hours under helium, the reaction was stopped by adjusting the pH to 1 with sulfuric acid and adding 2 ml of ethanol.

Isolation of Fatty Acids—Fatty acids were extracted directly from acid solution without prior alkaline hydrolysis as described in the previous paper (19). A small aliquot of the hexane extract containing the fatty acids was plated and counted immediately in a gas flow counter (Nuclear-Chicago Corporation) (26% efficiency). The remainder of the hexane was removed from the sample under a stream of nitrogen. After the residual fatty acids were dissolved in 0.5 ml of dry ether, the methyl esters of the fatty acids were formed by adding a few drops of a freshly prepared ethereal solution of diazomethane. After standing for 20 minutes at room temperature, the ether and excess diazomethane were blown off with nitrogen, and the methyl esters were dissolved in 0.5 ml of redistilled hexane and stored in the deep freeze for gas chromatographic analysis.

Gas Chromatographic Analysis—The radioactive fatty acids were identified by gas chromatography of their methyl esters with both an ethylene glycol succinate and an ethylene glycol adipate (6-ft U-tube, 4 mm, inside diameter) column with an argon detector. The radioactivity of the effluent peaks was measured by trapping the methyl esters as they emerged from the detector in a tube containing anthracene crystals coated with D.C. silicone oil 550, according to the method developed by Karmen and Tichy (23).

Materials—TPNH was obtained from the Sigma Chemical Company, CoA from Pabst Laboratories, and the malonic acid-2-C14 from Volk Radiochemical Company. Isovaleric and
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Fig. 1. Spectrophotometric evidence for the substitution of other acyl-CoA acceptors for acetyl-CoA in TPNH oxidation by the fatty acid-synthesizing system of adipose tissue. The complete system contained 50 μmoles of potassium phosphate buffer at pH 7.0, 2.5 μmoles of 2-mercaptoethanol, 0.05 μmole of malonyl-CoA, 0.05 μmole of acetyl-CoA or the appropriate acyl-CoA acceptor, and 0.2 μmole of TPNH in a final volume of 1.0 ml. The addition of 1 to 2 units of enzyme started the reaction. The decrease in absorbancy at 340 μm was followed.

Fig. 2. The separation of the methyl esters of the straight chain fatty acids from the methyl esters of the iso and the anteiso acids by gas chromatography with an ethylene glycol adipate column (6-ft U-tube 4 mm inside diameter), at 177° with an argon detector. The retention times for the methyl esters (---) were obtained from Table I by the method of Woodford and Van Gent.

Table I

Carbon numbers

<table>
<thead>
<tr>
<th>Normal saturated acids</th>
<th>Iso acids</th>
<th>Anteiso acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 (myristic)</td>
<td>13.55</td>
<td>14.7</td>
</tr>
<tr>
<td>15</td>
<td>14.5</td>
<td></td>
</tr>
<tr>
<td>10 (palmitic)</td>
<td>15.55 (Ref)*</td>
<td>16.7 (Ref)</td>
</tr>
<tr>
<td>17</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td>18 (stearic)</td>
<td>17.5 (Ref)</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>18.55 (Ref)</td>
<td>18.7 (Ref)</td>
</tr>
</tbody>
</table>

* Ref = model compounds available.

α-methylbutyric acids were obtained from Eastman Organic Chemicals, and isobutyric and isocaproic acids were obtained from Fisher Scientific Company.

The C₁₆, C₁₇, and C₁₈ iso acids and the C₁₇ and C₁₈ anteiso acids were generously provided by Dr. A. W. Weitkamp.

RESULTS

Spectrophotometric Experiments—The dependence of fatty acid synthesis on TPNH made it possible to follow the reaction by noting the decrease in absorbancy at 340 μm. Fig. 1 indicates that TPNH oxidation was largely dependent on the presence of acetyl-CoA in addition to malonyl-CoA. A comparison was made of the ability of propionyl-CoA, isobutyryl-CoA, isovaleryl-CoA, α-methylbutyryl-CoA, and isocapryl-CoA to substitute for acetyl-CoA in such spectrophotometric experiments. These experiments suggested that the acyl-CoA derivatives tested could substitute for acetyl-CoA in fatty acid synthesis and that the reaction products might be odd-numbered and branched chain fatty acids.

Identification of Fatty Acids by Gas Chromatography—Although separation of the iso and anteiso fatty acids synthesized might have been achieved by reverse phase column chromatography (24), the technique of gas chromatography coupled with the simultaneous recording of the radioactivity of the effluent peaks appeared to offer a more rapid and less cumbersome procedure for tentative identification of the reaction products. Fig. 2 shows that the methyl esters of myristic, palmitic, and stearic acids can be well separated from the esters of iso-C₁₆, C₁₈, and C₁₉ acids. The carbon numbers (chain length) for the iso and anteiso acids could be determined by direct measurement of the effluent on semilogarithmic paper. The products of the reaction with isobutyryl-CoA and isovaleryl-CoA were identified on both an ethylene glycol succinate and an ethylene glycol adipate column. The identifications were simplified in these experiments because no unsaturated fatty acids were synthesized by the soluble enzyme system. This fact was confirmed by the finding that the retention times of the enzymatic products were unchanged after catalytic hydrogenation.

Identification of Enzymatic Products—The results of an experiment in which isobutyryl CoA was incubated with malonyl-CoA-2-¹⁴C, TPNH, and enzyme are shown in Fig. 3. The principal product was 14-methylpentadecanoic acid (iso-C₁₆) with smaller amounts of iso-C₁₈ and iso-C₁₉ acids. The radioactivity was recorded as an integral record. There was a 10-second lag between the mass and radioactivity recordings with the instrument used in these experiments. There was no difficulty in determining that the radioactivity was associated with the iso-C₁₆ ester rather than the C₁₈ effluent peak. The iso-C₁₆ ester emerged from the column 1.93 minutes before the normal C₁₈ peak. Although the anteiso-C₁₆ model compound was not available, it can be seen from Fig. 2 that it would have been separated from the iso-C₁₆ acid.

The results of similar experiments with each of the acyl-CoA derivatives that were substituted for acetyl-CoA are summarized in Table II. It is seen that long chain fatty acids were synthesized in all cases, as measured by the incorporation of radioactivity from malonyl-CoA-2-¹⁴C. The percentage of malonyl-CoA-2-¹⁴C incorporated varied from 37 to 74%. This is equivalent to 4 to 10 μg of long chain fatty acid (calculated as palmitate). Odd-numbered acids were synthesized from propionyl-CoA, even numbered iso acids from isobutyryl-CoA and isocapryl-CoA, and odd-numbered acids from isovaleryl-CoA.
antio acids from a-methylbutyryl-CoA. Inasmuch as the positions of the branched methyl groups were deduced only on the basis of data obtained by comparison with known compounds by gas chromatography, these assignments should be considered tentative. Final confirmation of the structure of some of these compounds by mass spectrometry will be undertaken.

In experiments with less purified enzyme, small amounts of radioactive palmitate were synthesized in addition to the predominant branched chain acids (Fig. 3). A contaminating malonyl-CoA decarboxylase in the enzyme may have accounted for the palmitate synthesis with the cruder preparations by making acetyl-CoA available.

**DISCUSSION**

The purified soluble enzyme system from adipose tissue that synthesizes normal long chain fatty acids will also synthesize odd-numbered iso and anteiso acids. Therefore, the enzyme has a broad range of specificity. It is clear that the type of fatty acid synthesized by this enzyme system is determined by the structure of the acyl-CoA acceptor. In every case, only one acceptor unit entered into the final product and contributed only the methyl end of the long chain acid, corroborating the stoichiometry studies of the palmitate synthesis presented in the previous communication (19). This suggests that the initial condensation reaction of fatty acid synthesis occurs between a fatty acyl-CoA ester and malonyl-CoA and that the chain is lengthened by subsequent condensations with malonyl-CoA.

The fact that only small amounts of long chain odd-numbered and branched chain fatty acids are normally found in mammalian tissue probably reflects the low concentration of the appropriate acyl-CoA esters available for fatty acid synthesis compared to acetyl-CoA. Valine, leucine, and isoleucine could be the source of the isobutyryl-CoA, isovaleryl-CoA and a-methylbutyryl-CoA respectively (26–28). The activation of propionic acid by mammalian tissue to form propionyl-CoA has been reported (29), and this could be the source of the acyl-CoA acceptor units for long chain, odd-numbered fatty acids.

It is interesting that the syntheses described above stop with the formation of acids containing 15 to 17 carbon atoms, whereas the yeast enzyme system (6) stops with the formation of the C16-CoA ester. Apparently, the formation of free acids of this carbon chain length is characteristic of the rat epididymal enzyme system. The synthesis of the longer chain acids (C18 to C26) present in mammalian phospholipids may, like desaturation, be an enzymatic process associated with the particulate fraction of the cell.

**SUMMARY**

A 49-fold purified long chain fatty acid-synthesizing system from rat adipose tissue catalyzes the synthesis of the odd-numbered, iso and anteiso long chain fatty acids. The enzyme requires an odd-numbered, iso, or anteiso short chain fatty acyl coenzyme A derivative, in addition to malonyl coenzyme A and reduced triphosphopyridine nucleotide. The products of the reaction were tentatively identified by gas chromatography with simultaneous recording of radioactivity.

![FIG. 3. Gas chromatographic record of the products of the reaction of the enzyme (purified 49-fold) with isobutyryl-CoA as the acyl-CoA acceptor. The mass lines record the retention time of the long chain fatty acid; the lighter line is the integral record of the radioactivity of the effluent peaks and identifies the fatty acids synthesized during the reaction. Twenty micrograms of carrier methyl esters of iso-C16 and iso-C18 were added. An ethylene glycol adipate column at 177 °C was used.](image)

**TABLE II**

<table>
<thead>
<tr>
<th>Acyl-CoA acceptor</th>
<th>Amount incubated</th>
<th>Malonyl-CoA: acyl-CoA incorporated</th>
<th>Fatty acid synthesized*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl-CoA</td>
<td>0.20 (μmole)</td>
<td>60, 63, 74</td>
<td>C16 (C15, C18)</td>
</tr>
<tr>
<td>Propionyl-CoA</td>
<td>0.20 (μmole)</td>
<td>71</td>
<td>C16 (C15, C17)</td>
</tr>
<tr>
<td>Isobutyryl-CoA</td>
<td>0.24 (μmole)</td>
<td>64, 74</td>
<td>iso-C16, iso-C18</td>
</tr>
<tr>
<td>Isovaleryl-CoA</td>
<td>0.24 (μmole)</td>
<td>97, 55</td>
<td>iso-C14, iso-C15</td>
</tr>
<tr>
<td>α-Methylbutyryl-CoA</td>
<td>0.24 (μmole)</td>
<td>50, 55</td>
<td>anteiso-C13, anteiso-C17</td>
</tr>
</tbody>
</table>

* Major products as indicated; minor products listed in parentheses were present in less than 10% total quantity.

**REFERENCES**

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